Supplementary Methods

Bacterial strains and culture

H. pylori P12 and G27 are clinical isolates[1,2]. P12-GFP is a GFP-expressing derivate[3]. P12Δ*cag*PAI lacks the pathogenicity island[3]. G27Δ*fliG* is aflagellated[4]. G27Δ*motB* is non-motile[5]. P12 and derivates were a kind gift of Thomas F. Meyer. G27 and derivates were a kind gift from Karen Ottemann. Bacteria were grown on agar plates (GC agar, Becton Dickinson) supplemented with 10% Horse serum (Gibco), vancomycin (10 µg/mL), trimethoprim (5 µg/mL), and nystatin (1 µg/mL) under microaerobic conditions (85% N_2, 10% CO_2, 5% O_2) at 37°C. For the mutant bacteria, kanamycin (8 µg/mL) or chloramphenicol (4 µg/mL) was added as applicable.

PCR and Microarray

RNA was prepared from organoids, isolated gastric glands or isolated intestinal crypts according to manufacturer's recommendations (RNeasy Mini kit, Qiagen). cDNA was generated using reverse transcriptase (Promega). Quantitative real-time PCR was performed using SYBR green (BioRad) and CFX 384 Real Time system (BioRad). Results were calculated by using ΔΔCt method in Excel. Relative quantification was achieved by normalizing to the values of the GAPDH gene. Non-quantitative PCRs were performed using the same primers. Microarray (Affymetrix) analysis was performed on a genome-wide mRNA expression platform (Affymetrix Human Gene ST 2.0). Data was analyzed using the R2 web application (http://r2.amc.nl). The data has been deposited in the GEO database accession number GSE60557.

Primers used for PCR and qPCR:
MUC5AC 5'-CTTCTCAACGTTTGACGGGAAGC 5'-CTTGATCACCACCACCGTCTG-3'
MUC6 5'-GCCCCGGTATCTTCTCTCGG-3' 5'-ACACCTGCAGGGTGAGTACG-3'
PGC 5'-AGAGCCAGGCCTGCACCAGT-3' 5'-GCCCCTGTGGCCTGCAGAAG-3'
SST 5'-CTAGAGTTTGACCAGCCAC-3' 5'-GACAGATCTTCAGGTTCCAG-3'
LGR5 5'-TATGCCTTTGGAAACCTCTC-3' 5'-CACCATTCAGAGTCAGTGTT-3'
TNFRSF19 5'-CTGCTCATCCTCTGTGTCATCTATTG-3' 5'-GCCGTTGTACTGAATGTCCTGTG-3'
CD44 5'-AGATGGAGAAAGCTCTGAGC-3' 5'-GGTAATTGGTCCATCAAAGGC-3'
AXIN2 5'-ACTTCAAGTGCAAACTTTCG-3' 5'-GGGAAATGAGGTAGAGACAC-3'
IL-8 5'-ACACTGCGCCAACACAGAAAT-3' 5'-ATTGCATCTGGCAACCCTACA-3'
GAPDH 5'-GGTATCGTGGAAGGACTCATGAC-3' 5'-ATGCCAGTGAGCTTCCCGTTCAG-3'
CDX1 5'- GTGGCAGCGGTAAGACTC-3' 5'- GTTCACTTTGCGCTCCTTTGC-3'
CDX2 5'- AACCAGGACGAAAGACAAAT-3' 5'- GAAGACACCGGACTCAAG-3'
TFF1 5'- CCATGGAGAACAAGGTGAT-3' 5'- CACCAGGAAAACCACAATTC-3'
TFF2 5'- GACAATGGATGCTGTTTCG-3' 5'- GTAATGGCAGTCTTCCACAGA-3'

FACS

Single cells were sorted using MoFlo (Beckman Coulter). Isolated glands were prepared as described above, incubated in 10 mM EDTA and pipetted through a pointed glass pipette until most of the cells were single. Before FACS, cells were passed through a 20 µm cell strainer and washed with cold basal medium. Dead cells were excluded by propidium iodide staining. Single cells were gated by using forward scatter area versus forward scatter peak linear. This does not enrich for stem cells but was used to generate single cells.

Histology and Imaging

Organoids and tissue samples were fixed with 4% FA over night at 4°C. Paraffin sections and immunohistochemistry were generated as previously described[6]. For wholemount analysis, organoids were permeabilized with 0.3% Triton X-100 and stained in PBS 0.3% Triton, 1% BSA, 5% normal goat serum (with the exception of the anti-PGC antibody where BSA was omitted due to cross-reactivity). The following antibodies were used: MUC5AC (Vision biosystems 45M1), MUC6 (Santa Cruz T20 sc16914), PGC (Abcam ab9013), SST (Invitrogen 18-0078), E-Cadherin (BD Transduction labs 610405), p65 (Santa Cruz sc109). DNA was stained with DAPI (Molecular Probes). EdU staining was performed according to manufacturer's recommendations (Click-It, Invitrogen). Images were taken using standard or confocal microscopy (Nikon Eclipse E600, Leica DMIL, Leica SP5). Cell lineages we counted based on staining as performed previously[7]. Images were taken on Nicon Eclipse E600, and cells were counted in a blinded fashion in 7 images, containing each at least 1 organoid and a total of 500 cells per condition and staining.

Karyotyping

Metaphase spreads were generated after 24 h of colcemid treatment (0.1 μg/mL, Gibco). Organoids were removed from Matrigel and dissociated into single cells using trypsin. Cells were lysed with 0.075 M KCL and material fixed using methanol:acetic acid (3:1). For normal tissue, organoid lines from 2 patients (either 6 or 15 spreads) were karyotyped by the lab for cytogenetics in the Wilhelmina Children's Hospital in Utrecht using the software Case Data Manager from Applied Spectral Imaging. For tumor tissue, 7 metaphase spreads as depicted in figure 5B were counted from 1 organoid line.

Cell viability assay

Cell viability was analyzed using a luminescent cell viability assay (CellTiter-Glo, Promega) according to the manufacturer's recommendations. The assay is based on quantitation of the ATP present, which is an indication of the viable cells. Briefly, each 48 well of organoids was lysed in 200 μL reagents, mixed thoroughly and incubated for 10 minutes. 20 μL of the mix was transferred to a clean white-bottom 96 well plate and luminescence was measured. Data was compiled in Excel.

Supplementary References

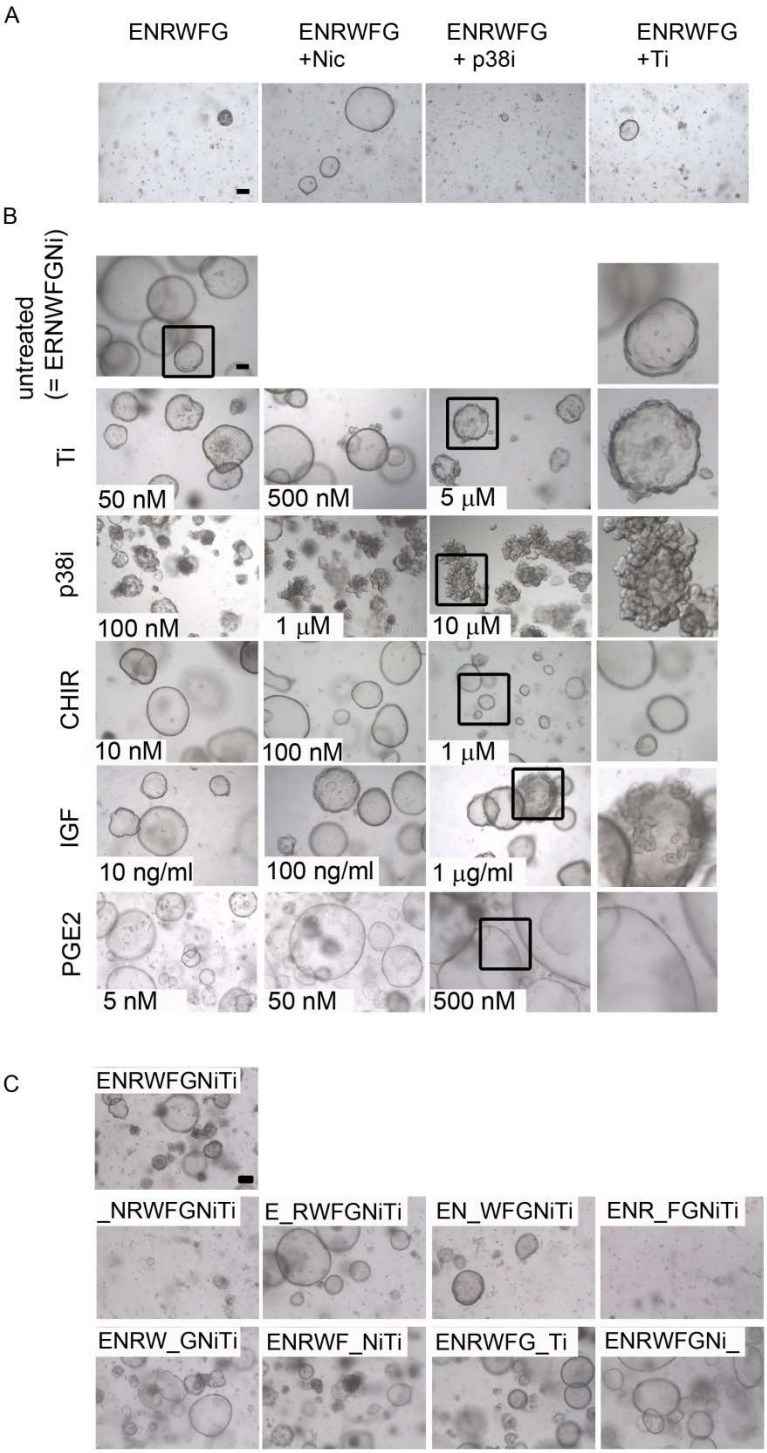
1. Odenbreit S, Till M, Haas R. Optimized BlaM-transposon shuttle mutagenesis of Helicobacter pylori allows the identification of novel genetic loci involved in bacterial virulence. Mol. Microbiol. 1996;20:361–373.
2. Censini S, Lange C, Xiang Z, et al. cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. Proc. Natl. Acad. Sci. 1996;93:14648–14653.
3. Wunder C, Churin Y, Winau F, et al. Cholesterol glucosylation promotes immune evasion by Helicobacter pylori. Nat. Med. 2006;12:1030–1038.
4. Lowenthal AC, Hill M, Sycuro LK, et al. Functional Analysis of the Helicobacter pylori Flagellar Switch Proteins. J. Bacteriol. 2009;191:7147–7156.
5. Ottemann KM. Helicobacter pylori Uses Motility for Initial Colonization and To Attain Robust Infection. Infect. Immun. 2002;70:1984–1990.
6. Batlle E, Henderson JT, Beghtel H, et al. β-Catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. Cell 2002;111:251–263.

7. Xiao C, Feng R, Engevik AC, et al. Sonic Hedgehog contributes to gastric mucosal restitution after injury. Laboratory Investigation 2012;93:96–111.

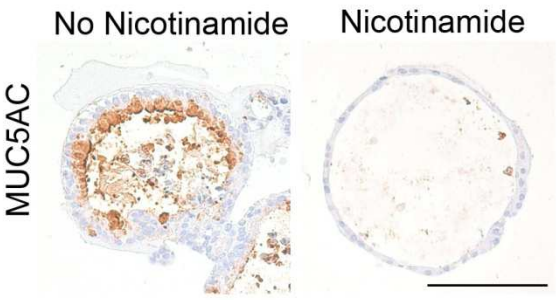
Supplementary Table 1: Genes regulated by *H. pylori* infection. Organoid cultures from 3 patients were infected with *H. pylori* at MOI 50 for 2 h. Genome-wide mRNA expression levels were analyzed with Affymetrix. Culture condition was ENRWFGNiTi.

	Fold change	P-value
CGB	14.56	0.0048
CCL20	14.37	0.0035
ICAM1	10.36	0.0086
IL8	9.52	0.0015
CYP3A5	3.94	0.0461
TNFAIP3	3.78	0.0208
CD83	3.44	0.0139
BIRC3	3.23	0.0132
IGKC	3.15	0.0214
CXCL1	3.15	0.0171
CHAC1	2.91	0.0487
TNF	2.88	0.0397
TNFRSF9	2.74	0.0183
NAMPT	2.69	0.0246
IL17C	2.61	0.0483
NFKBIA	2.50	0.0072
MIR146A	2.46	0.0173
MIR4320	2.35	0.0395
OR5M1	2.24	0.0089
ZC3H12A	2.20	0.0320
HIVEP2	2.16	0.0437
IRAK2	2.06	0.0437
C2orf16	-2.01	0.0268
RNU6-57	-2.09	0.0233
SNORA30	-2.33	0.0126

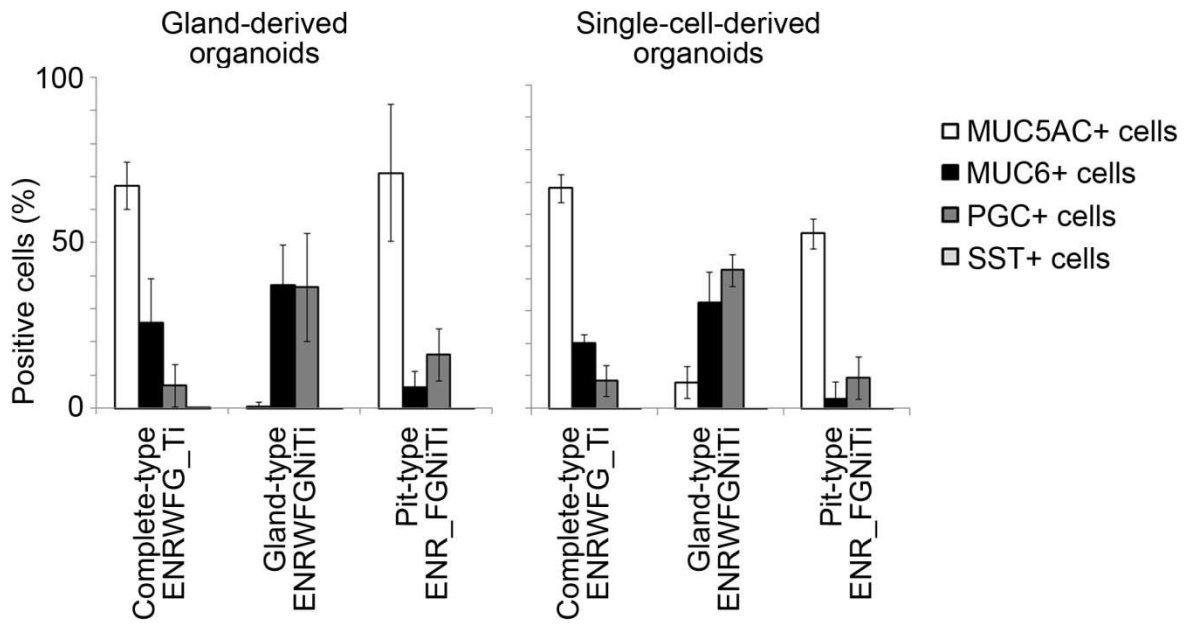
Supplementary figures



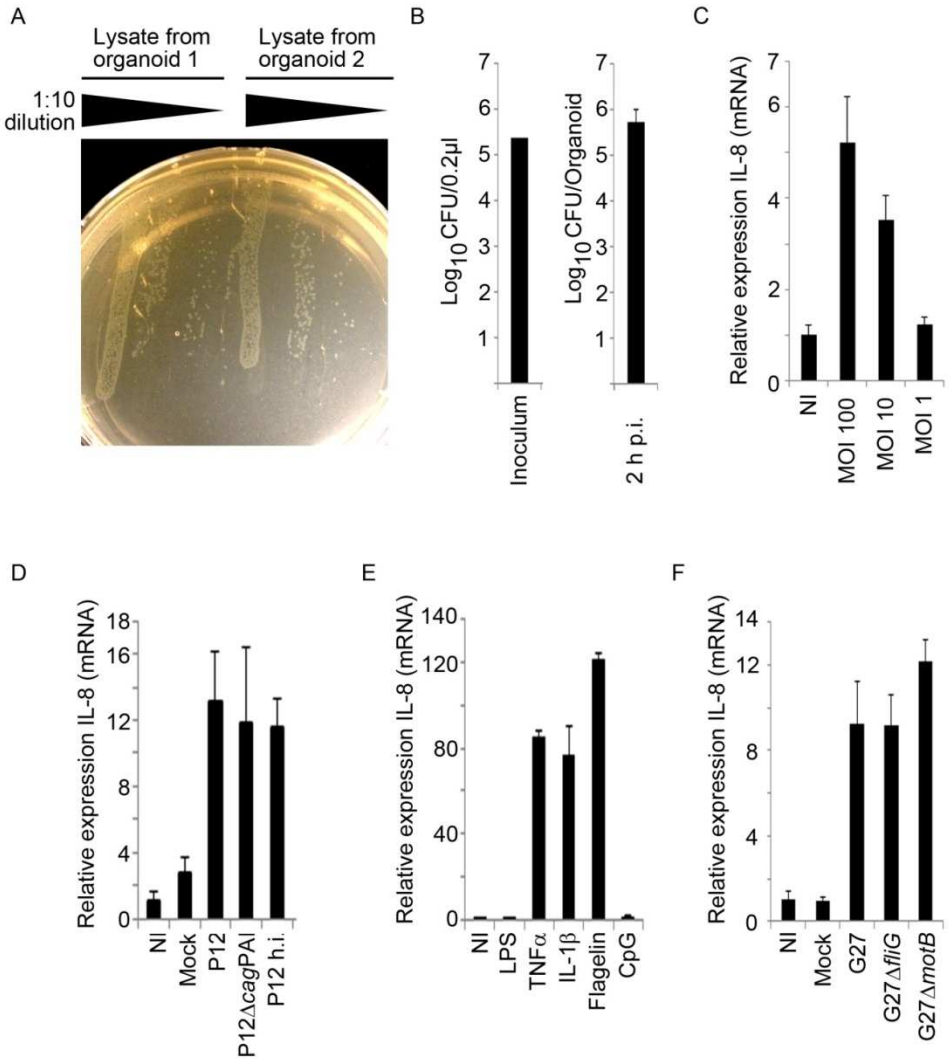
Supplementary figure 1: Establishing growth conditions for human gastric organoids. (A) Nicotinamide (Ni) supports organoid formation after 10 days. (B) Phenotypical changes and titration of growth factor concentration. High concentrations of TGFβ inhibitor (Ti), p38 inhibitor or IGF increase budding structures. CHIR decreases organoid size. PGE2 induces large cysts. (C) Formation of organoids in incomplete media. Wnt is essential for initial growth (ENR_FGNiTi). Cultures lacking other factors show already reduced organoid formation (compare Fig 1E). Scale bar 100 µm. The abbreviations for the medium ENRWFGNiTi refer to EGF, R-spondin1, Noggin, Wnt, FGF10, Gastrin, Nicotinamide and TGFβ-inhibitor.



Supplementary figure 2: Nicotinamide suppresses differentiation into mucous pit cell lineage. Images of stained paraffin sections of organoids. Cells were either grown in ENRWFG_Ti or ENRWFGNiTi. Scale bar 100 µm. The abbreviations for the medium ENRWFGNiTi refer to EGF, R-spondin1, Noggin, Wnt, FGF10, Gastrin, Nicotinamide and TGFβ-inhibitor.



Supplementary figure 3: Quantification of directed differentiation into gland and pit lineages. Cultures were either grown in ENRWFG_Ti to receive complete-type organoids (left panel), or grown in ENRWFGNiTi to receive gland-type organoids (middle panel) or first kept in ENRWFGNiTi for 10 days and subsequently in ENR_FGNiTi for 4 days to receive pit-type organoids (right panel). Organoids were fixed, embedded in paraffin and immuno-stained for the indicated markers. Positive cells and counterstained nuclei were blindly counted in seven images per condition. Each image contained at least one organoid and in total, at least 500 cells were counted per cell type. Bars represent averages of 7 images with standard deviation. The abbreviations for the medium ENRWFGNiTi refer to EGF, R-spondin1, Noggin, Wnt, FGF10, Gastrin, Nicotinamide and TGFβ-inhibitor. SST-positive cells are very rare. Most randomly taken images do not contain SST-positive cells, but they can be found in each condition.



Supplementary figure 4: *H. pylori* infection of human gastric organoids. (A) Bacteria can be cultured from infected organoids. 2 h after microinjection of bacteria into organoids, single infected organoids were picked, lysed and bacteria plated out in serial dilutions. (B) Viability of bacteria in the inoculum and after 2 h of infection in the organoids. Bacteria in the inoculum were quantified by plating out serial dilutions (left panel). Each organoid is injected with approximately 0.2 µl. After injection, bacteria were cultured back from the organoids as described in A and also quantified (right panel). Bar represents average of 8 organoids with standard deviation. CFU, colony forming units. (C) IL-8 mRNA induction depends on the MOI. Bacteria were injected at the indicated MOI and mRNA levels of IL-8 were assessed with qPCR. MOI, multiplicity of infection. (D) Induction of IL-8 mRNA does neither depend on the bacterial pathogenicity island (*cag*PAI) nor on viability of bacteria. Clinical isolate P12, its mutant P12Δ*cag*PAI and heat inactivated P12 were microinjected, organoids lysed after 2 h and induction of IL-8 was assessed by qPCR. (E) IL-8 mRNA is induced by TNFα, IL-1β and flagellin, but not by LPS and CpG. Components were added to the medium and IL-8 induction was assessed after 2 h by qPCR. (D) IL-8 induction does neither depend on *H. pylori* flagellin nor on bacterial motility. Clinical isolate G27, its aflagellated mutant G27Δ*fliG* and its nonmotile mutant G27Δ*motB* were microinjected and expression of IL-8 mRNA was assessed by qPCR. All mRNA was normalized to GAPDH housekeeping gene.

Supplementary material: Detailed protocol for human gastric organoid culture

Media

Reagent name	Supplier	Cat No	Stock solution	final concentration
HEPES	Invitrogen	15630-056		
Advanced DMEM/F12	Invitrogen	12634-028		
Matrigel, GFR, phenol free	BD	356231		
GlutaMAX-I	Invitrogen	35050-079	200 mM	2 mM
Penicillin/Streptomycin	Invitrogen	15140-122	10000/10000 U/mL	100/100 U/mL
B27	Invitrogen	17504-044	50 x	1x
N-Acetylcysteine	Sigma-Aldrich	A9165-5G	500 mM	1 mM
Murine recombinant EGF	Invitrogen	PMG8043	500 µg/mL	50 ng/mL
Human recombinant FGF10	Peprotech	100-26	100 µg/mL	200 ng/mL
TGFβi A-83-01	Tocris	2939	500 µM	2 µM
Nicotinamide	Sigma-Aldrich	N0636	1 M	10 mM
[Leu15]-Gastrin	Sigma-Aldrich	G9145	100 µM	1 nM
RHOKi Y-27632	Sigma-Aldrich	Y0503	10 mM	10 µM
Wnt3A conditioned medium	Stable cell line			50%
R-spondin1 conditioned medium	Stable cell line			10%
Noggin conditioned medium	Stable cell line			10%
Recovery Cell Culture Freezing Medium	Invitrogen	12648-010		

Isolation

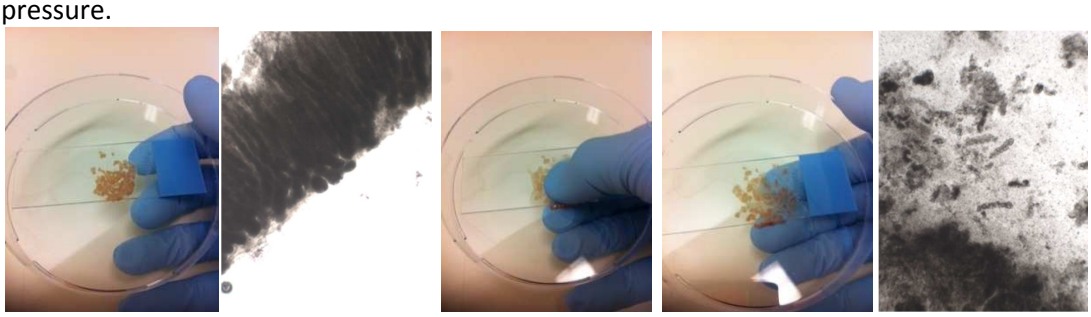
The following protocol can be used to generate organoids from tissue resection material or biopsies.

1. Pre-warm a 24-well plate one day before the isolation.
2. Let the Matrigel thaw on ice.
3. Prepare 500 mL cold chelating buffer (sterile distilled water with 5.6 mmol/L Na_2HPO_4, 8.0 mmol/L KH_2PO_4, 96.2 mmol/L NaCl, 1.6 mmol/L KCl, 43.4 mmol/L sucrose, 54.9 mmol/L D-sorbitol, 0.5 mmol/L DL-dithiothreitol, pH 7). Throughout the procedure, keep tissue in ice-cold chelating buffer as much as possible.
4. Place the tissue in chelating buffer in a 10 cm dish. Wash carefully by moving back and forth.
5. Place in a dry 10 cm dish. Carefully remove mucous and the muscle layer under a stereomicroscope using foreceps.
6. Wash the tissue in cold chelating buffer.
7. Place the tissue on a clean dry 10 cm dish. Cut in small pieces (about 5 mm) and place them into a 50 mL falcon tube. Immerse the pieces into 10 mL cold chelating buffer. Pre-wet a plastic 10 mL pipette and re-use the pipette throughout the procedure to minimize adherence of tissue to the plastic. Wash the tissue by vigorously pipetting up and down 10x. Let the pieces settle. Remove the supernatant, repeat washing until the supernatant is clear (5-10 times).
13. Add 20 mL chelating buffer 10 mM EDTA, incubate 10 min at room temperature.
14. Let the pieces settle. Pipette gently once up and down, let the pieces settle, discard supernatant.
15. Carefully transfer the tissue-pieces in the middle of a sterile 10 cm dish. Remove as much liquid as possible. Place a glass microscopy slide on top of the tissue. Under the microscope, tissue with intact glands can be observed. Apply pressure until the area around the tissue pieces

appears cloudy. Under the light microscope, glands are now visible in solution. The glandular structure is often not conserved over the following washing step (probably depending on the condition of the starting tissue) so at later stages, often parts of glands or even single cells will be visible.

16. Collect glands and tissue pieces in 30 mL of cold Advanced DMEM/F12. Let the large tissue fragments settle. Transfer the cloudy supernatant containing glands to two 15 ml falcon tubes. If preferred, at this stage the glands can be counted. Seed approximately 100 glands per 50 µL Matrigel per well of a 24 well plate. If glands have dissociated and counting is more difficult, seed a dilution row.
17. Centrifuge 5 min at 200 g and 4°C. Discard supernatant and resuspend with Matrigel (50 µl/well). In each well of a pre-warmed 24 well plate place a 50 µl drop of the Matrigel-cell mixture. Carefully transfer the plate to 37°C without disturbing the drop. Let it solidify for 10 minutes
18. Prepare warm medium with all growth factors.
19. Carefully overlay the Matrigel drop with 500 µL medium per well.
20. Transfer the plate to the incubator.
21. Refeed every 2-3 days (3 times per week).

Step 15 details: Tissue fragments in the dish and under the microscope before and after applying pressure.



Passage of the gastric organoid culture

Human gastric organoids are passaged every 2 weeks in a 1:5 ratio using mechanical dissociation.

1. Remove medium from Matrigel.
2. For 24 well plates, add 1 mL cold Advanced-DMEM/F12 per well. Break up the gel using a micropipette and transfer to 15 mL falcon tube
3. Narrow the end of a glass Pasteur pipette using fire. Wet the pipette in Advanced DMEM/F12. If the end is narrowed well, this pipette will take up medium slower than an un-narrowed pipette.
4. Take up the organoids in Advanced DMEM/F12 using the narrowed pipette. Pipette 10 x up and down, breaking up the organoids.
5. Centrifuge 5 min 200 g at 4°C.
6. Carefully discard supernatant as much as possible and resuspend with Matrigel. In each well of a pre-warmed 24 well plate place a 50 µL drop of the Matrigel-cell mixture. Carefully transfer the plate to 37°C without disturbing the drop. Let it solidify for 10 minutes
7. Prepare warm medium with all growth factors.
8. Carefully overlay the Matrigel drop with 500 µL medium per well.
9. Transfer the plate to the incubator.
10. Refeed every 2-3 days (3 times per week).

Long term storage

Organoids can be frozen and stored in liquid nitrogen.

1. Disrupt the organoids as for passaging using the Pasteur pipette.
2. Dispense the fragments in cold Recovery Cell Culture Freezing Medium (1 mL/well) and place them in 1,5-mL cryotubes.

3. Freeze down over night in a -80°C freezer in a cryo-freezing container (Mr. Frosty, Nalgene).
4. Cells can then be transferred to liquid nitrogen.
5. For thawing, warm the cryotube at 37°C and suspend the cells in 10 mL Advanced DMEM/F12.
6. Centrifuge the cells for 5 min at 200 g and 4°C.
7. Resuspend the pelleted cells in 50 µL Matrigel and place a 50 µL drop in the center of a well of a pre-warmed 24 well plate. Carefully place the plate in the incubator and let the Matrigel solidify at 37°C for 10 minutes.
8. Overlay the Matrigel drop with 500 µL of prewarmed medium containing all growth factors including RHOKi.